

# Genetic Analysis of Historical Human Remains – What ancient Bones can tell if one asks the right Questions...

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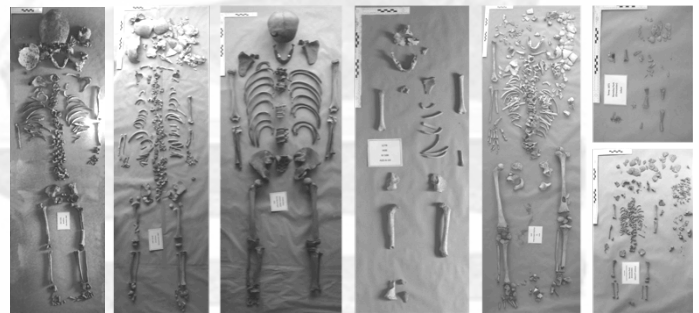
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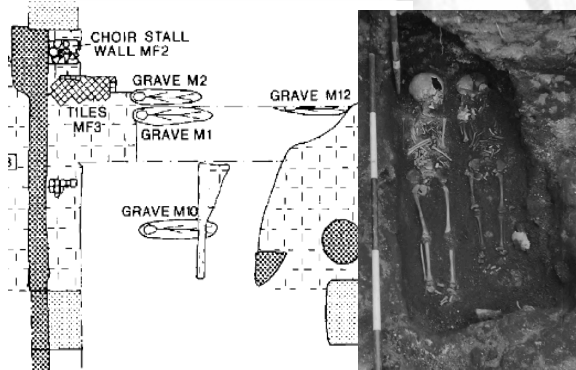
Ancient DNA analysis of historical human remains explores similar questions, utilizing similar and frequently the same methodology as applied in the forensic human identification context, while doing so under extreme conditions regarding DNA content, degree of degradation and presence of inhibitors. Consequently, improvements of methods and procedures in one of the areas will inform the other and vice versa. This presentation features examples of our current Ancient DNA based research on historical remains from the collection of the Gloucester City Museum.

In the forensic context, the ability to sex skeletal remains reliably is of utmost importance in order to ascertain the identity of an individual. Contrary to the assumption that sex determination in case of sub-adults skeletal remains it is impossible or unreliable (Cardoso & Saunders 2008, Wilson et al. 2008), it is indeed possible to sex juvenile skeletal remains and findings regarding sexual dimorphisms in the sub-adult skeleton have been published for more than a century (Thomson 1899, Boucher 1957, Sundick 1977). A variety of methods to determine the sex of immature human skeletal remains reliably have been published and subsequently tested by experienced anthropologists (e.g. Hunt 1990, Mittler and Sheridan 1992, Scheuer 2002, Sutter 2003, Wilson et al. 2008, Cardoso & Saunders 2008).

To evaluate the reliability of these methods when applied by researchers with limited experience in osteological analysis, six previously published methods assessing different skeletal areas (Boucher 1957, Black 1978, Weaver 1980, Schutkowski 1993, Loth & Henneberg 2001, Rogers 2009) were tested in use by students with initial osteological training. The selected methods were utilized to determine the sex of seven sub-adult skeletal individuals from the Gloucester Museum collection. Results of the osteological analysis were compared to molecular sex determination based on amplifications of both Amelogenin (Sullivan et al. 1993) and SRY (Santos et al. 1998).



Sub-adult skeletons selected for the study (infans I to juvenis). Left to right: GF B5 and GF B6 (16<sup>th</sup> cent.), BM A8206 (roman), 5/78 HSR A22-A-14, T 1871 4th Century (top) and T 1871 (bottom).



Potential 16<sup>th</sup> century family burial inside the choir of a priory church: Successive burial layers. Upper layer: adult male and female M1 and M2, middle layer: adult male M3, bottom burials: 2 infants M4 and M5 (see picture, Ferris 2001).

As in the forensic context, identification of individuals as well as kinship analysis involving group burials or across entire grave collectives are applications for DNA analysis-based methodology in the study of historical skeletal remains. The example here is a collective burial, excavated from the choir of a priory church in England, which based on the relative location of the individual remains indicates a potential family burial: The group includes five individuals which were buried in three consecutive horizons, with two infants in the bottom layer, followed by a male adult and a two further adults (male and female) in the top layer (Ferris 2001).

Analysis here utilized an STR multiplex of own design based on published medium amplicon primer sets (Kimpton et al 1993), combined with molecular sex determination (Amelogenin and SRY). In a second phase this will be followed by further amplifications in multi- and singleplex reactions.

For DNA analysis in both studies, ca. 1g of bone from rib- or long bone fragments was sampled, surfaces were removed, the sample decontaminated and ground to a fine powder. Initially, DNA was extracted from 0.1g bone following a Chelex protocol with subsequent precipitation (Schmerer in prep.) after a 70h decalcification in 0.5M EDTA (pH 8.3). Initial amplifications showed the presence of remaining inhibitors in extracts from the historical remains, which is a common problem when amplifying ancient DNA (Höss & Pääbo 1993, Schmerer et al. 1999). In the first phase, ribs were sampled whenever possible to minimize invasiveness of sampling. To improve outcomes, the second phase utilizes 0.3g of samples with higher content of compact bone and a replacement of EDTA solution after 24h and a decalcification for 96h (Schmerer 2003), followed by silica column-based extraction (Omega Biotek 2013).

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References: Black TK (1978) Am J Phys Anthropol 48: 77-82, Boucher BJ (1957) Am J Phys Anthropol 15(4): 581-600, Cardoso HFV & Saunders SR (2008) Forensic Sci Int 178: 24-29, Ferris LM (2001) Trans. Bristol & Gloucestershire Archaeol Soc 119: 95-146, Höss M & Pääbo S (1993) Nucleic Acids Res 21(16): 3913-3914, Hunt DR (1990) J Forensic Sci 35(4): 881-885, Kimpton CP, Gill P, Walton A, Urquhart A, Millican ES, Adams M (1994) PCR Methods Appl 3(1): 13-22, Loth SR & Henneberg M (2001) Am J Phys Anthropol 115(2): 179-186, Mittler DM & Sheridan SG (1992) J Forensic Sci 37(4): 1068-1075, Omega Biotek (2013) E.Z.N.A.® Blood DNA Mini Kit. Manual Revision May 2013, Rogers TL (2009) Am J Phys Anthropol 140: 148-154, Santos FR, Pandya A & Tyler-Smith C (1998) Nature Genetics 18(2): 103, Scheuer L (2002) Am J Phys Anthropol 119(2): 189-191, Schmerer WM, Hummel S & Herrmann B (1999) Electrophoresis 20(8): 1712-1716, Schmerer WM (in prep.), Schmerer WM (2003) Methods Mol Biol 206: 57-61, Schutkowski H (1993) Am J Phys Anthropol 90(2): 199-205, Sullivan KM, Mannucci A, Kimpton CP & Gill P (1993) BioTechniques 15: 637-641, Sundick RI (1977) J Forensic Sci 22(1): 141-144, Sutter RC (2003) J Forensic Sci 48(5): 927-935, Thomson A (1899) J Anat Physiol 33(3): 359-380, Weaver DS (1980) Am J Phys Anthropol 52(2): 191-196, Wilson LA, Macleod N & Humphrey LT (2008) J Forensic Sci 53(2): 269-278.